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Fermentation Characteristics of Rice Crop Residue-Based Silage Treated by Epiphytic and Commercial LAB

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ABSTRAK

Dua percobaan dilakukan untuk mengetahui pengaruh penambahan inokulan bakteri asam laktat (BAL) yang berasal dari rumput raja dan inokulan komersil *L. plantarum* terhadap karakteristik fermentasi dan kecernaan silase berbasis sisa tanaman padi. Percobaan 1, campuran sisa tanaman padi (TP), ampas tahu (AT) dan onggok (OG) dengan rasio 80 : 10 : 10 berdasarkan bahan kering (BK) digunakan sebagai digunakan sebagai bahan silase. Empat perlakuan silase, yaitu (A) TP + AT + OG sebagai kontrol; (B) TP + AT + OG + 2% (v/b) inokulan BAL berasal dari rumput raja; (C) TP + AT + OG + 3% (v/b) inokulan BAL berasal dari rumput raja; (D) TP + AT + OG + 2% (v/b) inokulan *L. plantarum*. Percobaan 2, sebanyak 6 ekor kambing kacang digunakan dalam rancangan bujur sangkar Youden 6 × 3 dan diberi pakan rumput gajah, jerami padi, dan silase berbasis sisa tanam-an padi. Hasil penelitian menunjukkan bahwa silase B, C, dan D mengandung protein kasar (PK) lebih tinggi dibandingkan silase A. Konsentrasi asam laktat lebih tinggi (P<0,01) pada silase B dan C dibandingkan silase D. Nilai pH pada silase A lebih tinggi (P<0,05) dibandingkan silase dengan perlakuan inokulan BAL. Kecernaan BK, bahan organik, dan PK silase sisa tanaman padi lebih tinggi (P<0,01) dibandingkan jerami padi. Disimpulkan bahwa penambahan BAL epifit dari rumput raja pada sisa tanaman padi menghasilkan kualitas fermentasi yang lebih baik dibandingkan inokulan *L. plantarum*.

Kata kunci: silase, sisa tanaman padi, bakteri asam laktat, kecernaan

ABSTRACT

Two experiments were conducted to investigate the effects of addition of lactic acid bacteria (LAB) inoculant from king grass and a commercial inoculant of *L. plantarum* on fermentation characteristics and nutrient digestibility of rice crop residue-based silage. In experiment 1, mixture of rice crop residue (RC), soybean curd residue (SC) and cassava waste (CW) in a 80 : 10 : 10 (on dry matter basis) ratio was used as silage material. Four treatments silage were (A) RC + SC + CW as a control; (B) RC + SC + CW + LAB inoculant from king grass (2%, v/w) ; (C) RC + SC + CW + LAB inoculant from king grass (3%, v/w); (D) RC + SC + CW + *L. plantarum* inoculant (2%, v/w). In experiment 2, six Kacang goats were used in a 6 × 3 Youden square experiment and fed elephant grass, rice straw, and rice crop residue-based silage. The results showed that crude protein (CP) content in silages B, C, and D was slightly higher than silage A. Lactic acid concentration was significantly higher (P<0.01) in silages B and C compared to silage D. The pH value of control silage (A) was higher (P<0.05) than silage treated with LAB inoculant. Rice crop residue-based silage had higher (P<0.01) digestibility of dry matter (DM), organic matter (OM), and CP than rice straw. It was concluded that addition of epiphytic LAB inoculant from king grass to rice crop residue resulting in a good fermentation quality of silage compared to addition of *L. plantarum* inoculant.

Key words: silage, rice crop residue, lactic acid bacteria, digestibility

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INTRODUCTION

In line with the shortage of grasses as a result of the change of land for plantations, housing and industry, it would require many efforts to utilize agricultural and food processing industry residues as feed source for ruminant. Recently, there is growing interest in the use of agricultural and food processing industry residues as silage materials. Ensiling is regarded as a good forage preservation method which has been widely used for many years, since fermentation by some microbes is an effective way to improve the digestibility, palatability and nutritive value of straws (Nishino *et al*., 2004; Gao *et al*., 2008). Ruminants fed silage may have a number of beneficial effects due to conversion lactic acid into propionic acid which is the precursor of gluconeogenesis (McDonald *et al*., 1987) and probiotic effect of the lactic acid bacteria (LAB) used in inoculants (Weinberg *et al*., 2004).

The rice crop residue is lower part of rice crop after being harvested and left at rice field. These residues are abundantly available, and most of them are not be used as livestock feeds and is burned after dried thus causing air pollution. Takahashi *et al.* (2005) revealed that rice crop residue is potential to be preserved as silage and used as ruminant feeds. However, successful ensiling of rice straw is difficult due to its hollow stem, low water soluble carbohydrates (WSC) and less epiphytic LAB (Cai, 2006). Sugar-rich materials are commonly used as effective additives for ensiling crops that have low WSC. Cassava waste is a solid residu produced from cassava powder with its primary component of starch. Besides, soybean curd residue is a residue resulted from tofu processing and containing high crude protein *i.e.* 21.8±4.5%, and therefore it can be used as protein source for livestock (Santoso & Hariadi, 2009).

The LAB play an important role in silage fermentation and influence silage quality. Under natural circumtances, LAB grows as epiphytic bacteria, however, the population of LAB is usually low and variable with standing crops (Muck, 1990). Thus, addition of LAB inoculant is needed to improve silage quality (Bureenok *et al*., 2006). In the previous studies, Yahaya *et al*. (2004); Bureenok *et al*. (2005); Bureenok *et al.* (2006) stated that tropical and temperate forages ensiled with addition of epiphytic lactic acid bacteria inoculant resulting good fermentation quality compared to commercial inoculant. Santoso *et al.* (2009) concluded that fermentative quality of grass silage treated with epiphytic LAB prepared from king grass was better than those prepared from elephant grass. Similar result was reported in other experiment of Antaribaba *et al.* (2009); Santoso *et al*. (2011) that king grass silage with addition of epiphytic LAB had good fermentation quality compared to control silage, as indicated by high lactic acid content and *in vitro* nutrient digestibility, and low N-amonia concentration. Wang *et al*. (2009) concluded that the effect of LAB from forage crop may be comparable or even better than commercial bacterial culture, because the commercial bacterial does not grow well on the target crop.

Addition of LAB inoculant in silage materials could improve fermentation quality of silage and nutrient digestibility. Thus the experiment was carried out to evaluate the nutritive value and fermentation characteristic of rice crop residue-based silage treated with addition of epiphytic LAB inoculant from king grass extract and a commercial inoculant of *L. plantarum*.

MATERIALS AND METHODS

Silage Materials

Fresh rice crop residue (*Oryza sativa* var. Mygongga) was obtained from rice field area at Prafi District, Manokwari regency. Soybean curd and cassava waste were collected from small-scale food industry located at Prafi District. King grass was harvested at 50 days of regrowth defoliation from the experimental field of Faculty of Animal Science, Fishery and Marine Science, State University of Papua in Manokwari.

Inoculants Preparation

The epiphytic LAB inoculant was prepared according to modified of Bureenok *et al*. (2006) procedure as previously described by Santoso *et al.* (2009); Santoso *et al*. (2011). The inoculant was made using 220 g of fresh king grass or fresh rice crop residue, which was macerated in 1000 ml of distilled water using a high-speed blender for 4 min. The macerated was filtered through two layers of cheesecloths, and 600 ml of filtrate was collected in Erlenmeyer glass containing 18 g of glucose. The filtrate was mixed well and incubated anaerobically for 48 h at 30 ºC. At the end of 48 h, inoculant was used as source of LAB. The number of LAB inoculant was counted before the experiments by using de Man, Rogosa, and Sharpe (MRS) which were incubated for 3 days at 35 °C (Bureenok *et al*., 2006). The commercial *L. plantarum* was provided by Laboratory of Food Technology, Gadjah Mada University and then prepared by incubation in MRS broth (Difco Laboratories) at 30 °C for 48 h. LAB strain in king grass extract was isolated, purified and identified using a commercial kit (API 50 CH, bioMérieux, Inc. Durham, NC, USA) according to the manufacturer's recommendation.

Isolation and Purification of LAB

Briefly, 100 ml of dilution from first step preparation was then placed on MRS agar in triplicates and kept in anaerobic condition using anaerobic jar. They were incubated at 30 °C until colonies were visible. A single colony from above procedure was chosen and they were sub cultured for two or three times to get purified bacteria. Finally, the purified bacteria were used for identification strain of LAB.

Experiment 1 (Silages Preparation and Treatments)

Fresh rice crop residue was chopped with a domestic cutter to approximately 2–3 cm lengths. The chopped rice crop residue (RC), soybean curd residue (SC) and cassava waste (CW) were thoroughly mixed in a 80 : 10 :

10 ratio (on DM basis) and used as silage material. Four treatments silage were (A) RC + SC + CW as a control; (B) RC + SC + CW + LAB inoculant from king grass (2%, v/w); (C) RC + SC + CW + LAB inoculant from king grass (3%, v/w); (D) RC + SC + CW + *L. plantarum* inoculant (2%, v/w). The inoculants were sprayed onto silage material and subsequently mixed by hand before packing into silos. About 1.5 kg of silage materials were packed into plastic silos (295 × 495 × 0.06 mm) and stored in room temperature (± 28 °C) for 30 days. Each treatment was prepared in 9 replications and opened after 5, 10, and 30 days of ensiling. Samples were collected for preparation of silage extract and sample analyses.

Experiment 2 (*in Vivo* Nutrient Digestibility)

Six Kacang goats (an indigenous breed found in Indonesia) with an initial body weight (BW) of 16.3±1.8 kg were used in a 6 × 3 Youden square experiment. Goats were housed in six individual metabolism cages that facilitated separate collection of feces and urine. The experimental diets consisted of (A) elephant grass; (B) rice straw and (C) rice crop residue-based silage supplied twice a day (08:00 and 16:00 h) at a maintenance level of DM intake (66 g DM/kgBW$^{0.75}$/day) as recommended by Kearl (1982). Fresh water and a salt lick were available *ad libitum*. Before the start of the experiment, goats were dewormed with 2.5 mg/kg BW of Ascamex. Each period of the experiment lasted 13 days and was comprised of 8 days for diet adaptation and 5 days for digestion trial study. Total fecal excretions by each goat were collected and weighed. Individual feed refusals if any, were collected, weighed daily and samples bulked for analyses. Feces were sub-sampled, bulked and stored at −15 °C for subsequent analyses. Chemical analyses of feces samples were done with six replication of each treatment. Goats were weighed full at the beginning and the end of each period.

Chemical Analyses

Dried samples were used to determine DM, ash and crude protein (CP) according to procedure of AOAC (2005). Procedure of Van Soest *et al.* (1991) was used to determined concentrations of NDF, acid detergent fiber (ADF) and acid detergent lignin (ADL). NDF was determined without the use of α-amylase and sodium sulfite.

A 20 g of silage was macerated with 70 ml of distilled water and stored at 4 °C for 24 h. It was then homogenized for 15 min by using a shaker and filtered through a Whatman No. 1542 filter paper. The filtrate was used for determine of pH, VFAs, lactic acid and NH_3-N. The pH value was determined using a pH meter (Hanna Hi 9025). Concentrations of individual VFAs were analyzed using a gas chromatography (Varian CP-9002 GC, Shimadzu, Japan) equipped with flame ionization detector (FID) and stainless steel column (1500 mm × 3 mm i.d). The pressure of nitrogen was 1.25 kg/cm^2. The temperature of injector oven, column oven and detector were 220, 130 and 220 °C, respectively. Concentrations of lactic acid and NH_3-N were analyzed according to method of Barker & Summerson (1941); Chaney & Marbach (1962), respectively. Fleigh Point of the silage were calculated according to formulae as follows : Fleigh Point = 220 + (2 × DM% - 15) - (40 × pH), where Fleigh Point denote values between 85 and 100, very good quality; 60 and 80, good quality; 55 and 60, moderate quality; 25 and 40, satisfying quality; < 20, worthless (Ozturk *et al*., 2006).

Statistical Analysis

Data of fermentation characteristics of silage were subjected to analysis of variance for completely randomized design using GLM procedure of SAS (SAS Institute Inc., Cary, NC) with the model :

$$Y_{ij} = \mu + \alpha_i + \varepsilon_{ij}$$

where Y_{ij} is the observed value for the *i*th treatment and *j*th replicate, μ is overall mean, α_i is treatment effect for the *i*th treatment, εij is the random error associated with Y_{ij} experimental unit. Data of nutrient digestibility were analyzed using following the model :

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \gamma_k + \varepsilon_{ijk}$$

where Y_{ijk} is the observed value for the *i*th treatment, *j*th animal, and *k*th period, μ is overall mean, α_i is treatment effect for the *i*th treatment, β_j is animal effect for the *j*th animal, γ_k is period effect for the *k*th period, ε_{ijk} is the random error associated with Y_{ijk} experimental unit. Duncan's multiple range test was used to identify significant differences between means.

RESULTS AND DISCUSSION

Characteristics of King Grass Extract

After 48 h of anaerobic incubation, LAB number in king grass extract increased by 10 fold and followed by decreasing pH value from 6.81 to 3.29 (Table 1). This result indicate that LAB produced high lactic acid concentration during incubation which resulted in low pH value. This result is consistent with previous studies by Antaribaba *et al*. (2009); Santoso *et al*. (2009); Santoso *et al.* (2011) that pH value in king grass extract declined from average of 6.62 to 3.40 after 48 h of incubation at 30 °C. Trend declined pH value in extracts of grass and legume after 48 h of incubation have been also reported by Bureenok *et al*. (2006) and Wang *et al*. (2009). Two strains LAB found in king grass extract were *Lactobacillus plantarum* and *Lactobacillus brevis*.

Table 1. Changes of pH value and LAB number in king grass extract before and after 48 h of incubation

	Before incubation	After incubation
pH value	6.81	3.29
LAB (× 10^{10}cfu/ml)	0.30	3.00

Chemical Composition and Fermentative Quality of Silage

The DM content of silages was lower than the value of 30% for ideal silage as suggested by Chamberlain & Wilkinson (1996). This was due to rice crop residue mixed by soybean curd residue and cassava waste which has high moisture content *i.e*. 86.9% and 82.0%, respectively (Table 2). Relatively high OM content (P>0.05) was observed in silage B and C than control silage (A). This may be attributed to carbohydrate degradation to organic acids *i.e.* acetic, propionic and butyric acids in both silages was lower than silage A, as supported by total VFA concentration in both silages. A relative high CP content (P>0.05) was observed in silage treated with epiphytic LAB (B and C) and *L. plantarum* (D) could be due to low degradation of CP to amino acids and ammonia during ensiling. This result is consistent with NH_3-N concentration in all silages (Table 3). Silages B, C and D tended have lower (P=0.08) ADF concentration than the control silage. It has been reported that activity of cellulase and hemicellulase enzymes was high during ensilage (Yahaya *et al*., 2004). Similar results were also reported in other experiments using guinea grass and king grass silages (Ando *et al*., 2006; Antaribaba *et al*., 2009; Santoso *et al*., 2009; Santoso *et al*., 2011).

The fermentation characteristics of rice crop residue-based silage treated with LAB from king grass and *L. plantarum* inoculants are presented in Table 3. Lactic acid production in silage C and B was higher (P<0.01) than silage D and A. As compared to control silage (A), lactic acid concentration increased by 41.3%, 50.6%, and 24.8% respectively for silage B, C, and D. High lactic acid concentration in silage treated with epiphytic LAB (B and C) and *L. plantarum* inoculant (D) followed by lower (P<0.01) pH value than control silage (A). McDonald *et al.* (1991) stated that reducing pH silage prevented the growth of undesirable microbes *e.g*. listeria, clostridia, enterobacteriaceae and moulds. However, pH value of all silage are still above than ideal silage pH of 4.0 to 4.5 as suggested by Chamberlain & Wilkinson (1996). Higher pH value in silage could be attributed to limited of water soluble carbohydrate content, thus resulted in low lactic acid concentration produced by LAB. This result was similar with previoulsy studied by Cao *et al*. (2010) whole crop rice silage added with *L. plantarum*

Table 2. Chemical composition (% of DM) of rice crop residue-based silage

	Silages				SEM	P
	A	B	C	D		
Dry matter (DM)	20.4^{B}	20.5^{B}	21.6^{AB}	22.4^{A}	0.30	<0.01
Organic matter	82.0	81.8	82.2	82.2	0.25	0.66
Crude protein	5.3	5.7	5.7	6.0	0.20	0.19
NDF	69.7	66.7	67.8	66.5	1.10	0.20
ADF	60.8	57.7	57.8	56.7	0.99	0.08

Note: Means in the same row with different superscript differ significantly (P<0.01). A=rice crop residue + soybean curd residue + cassava waste (80:10:10, DM basis); B=rice crop residue + soybean curd residue + cassava waste (80:10:10, DM basis) + LAB inoculant from king grass (2%, v/w); C=rice crop residue + soybean curd residue + cassava waste (80:10:10, DM basis) + LAB inoculant from king grass (3%, v/w); D=rice crop residue + soybean curd residue + cassava waste (80:10:10, DM basis) + *L. plantarum* (2%, v/w). NDF: neutral detergent fiber; ADF: acid detergent fiber; SEM: standard error of the mean.

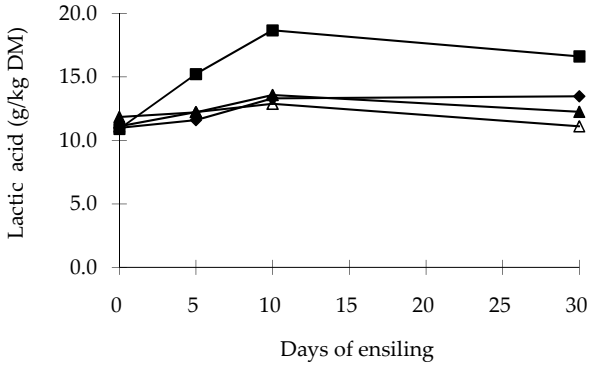
Table 3. Fermentation characteristics of rice crop residue-based silage

	Silages				SEM	P
	A	B	C	D		
pH	5.10^{a}	4.67^{b}	4.61^{b}	4.72^{b}	0.09	0.02
Lactic acid (g/kg DM)	31.0^{C}	43.8^{A}	46.7^{A}	38.7^{B}	1.01	<0.01
NH3-N(g/kg DM)	4.3	3.9	3.2	3.1	0.36	0.14
Acetic acid (g/kg DM)	9.93^{b}	18.9^{a}	19.0^{a}	16.6^{ab}	2.05	0.04
Propionic acid (g/kg DM)	1.1^{B}	3.6^{A}	2.9^{AB}	2.9^{AB}	0.38	<0.01
Butyric acid (g/kg DM)	3.5	3.3	3.1	2.6	0.64	0.81
Total VFA(g/kg DM)	14.5^{b}	25.9^{a}	25.0^{a}	22.2^{ab}	2.51	0.04
Fleigh Point	45.7	59.3	63.8	56.7	4.14	0.07

Note: Means in the same row with different superscript differ significantly ($^{a-b}$ P<0.05; $^{A-B}$P<0.01). A=rice crop residue + soybean curd residue + cassava waste (80:10:10, DM basis); B=rice crop residue + soybean curd residue + cassava waste (80:10:10, DM basis) + LAB inoculant from king grass (2%, v/w); C=rice crop residue + soybean curd residue + cassava waste (80:10:10, DM basis) + LAB inoculant from king grass (3%, v/w); D=rice crop residue + soybean curd residue + cassava waste (80:10:10, DM basis) + *L. plantarum* (2%, v/w). VFA: volatile fatty acids; SEM: standard error of the mean.

had higher lactic acid concentration than molasses and control silage. Chamberlain (1987) stated that high lactic acid concentration of silage has beneficial for ruminant because *Megasphaera elsdenii* metabolizes lactic acid to propionic acid and then is used as gluconeogenesis precursor. Change in lactic acid concentration during fermentation of rice crop residue based silage is shown in Figure 1. A rapid increase in lactic acid concentration occured in silage C during 10 days of ensiling. Furthermore, lactic acid concentration in all silages was slightly declined up to 30 days of ensiling.

Concentrations of NH_3-N and butyric acid were similar (P>0.05) among silage treatments. Silages B and C had higher (P<0.01) concentration of acetic acid than silage A. This result indicates that activity of heterofermentative LAB in both silages B and C was higher than in silage A. McDonald *et al*. (1991) revealed that during ensiling, hexose is fermented to lactid acid and other products *i.e*. ethanol and acetic acid. In our research, although there was no difference among silages on butyric acid concentration, silage A had relative higher butyric acid than other silages. That results indicate that clostridia bacteria was more active in control silage (A) than silage treated with epiphytic LAB or *L. plantarum* inoculant. According to Chamberlain & Wilkinson (1996), secondary fermentation occurs insufficient acid is produced by the primary fermentation to reduce the pH to below a critical level of about 4.5. The bacteria responsible for secondary fermentations are mainly the clostridia. These bacteria may convert lactic acid to butyric, or the may degrade proteins, peptides and amino acids to amines and ammonia. McDonald *et al.* (1987) also reported that butyric acid is produced by saccharolytic clostridia *i.e*. *Clostridium butyricum*. The total VFA concentration tended to decrease in silages added with LAB inoculant than control silage. The result indicates that additon of LAB inoculant to rice crop residue could improve fermentative quality of silage. Chamberlain & Wilkinson (1996) stated that the VFA comprise acitic acid, propionic acid, butyric acid and other acids. The production of these acids is reflection of an inefficient fermentation or of secondary fermentation of lactic acid to butiryc acid and degradation of amino acids to ammonia with the production of amino acid from skeleton of the amino acid. In the present study, the proportion of VFA to total acid was 31.9%, 37.2%, 34.9%, and 36.5%, respectively for silage A, B, C, and D. The result indicates that fermentation of silage A was more efficient than silage B, C, and D. However, the values found in this study are still above than ideal value of 20% as recommended by Chamberlain & Wilkinson (1996).



Fleigh point in silage C was relatively higher than other silages, suggesting that silage treated with epiphytic LAB prepared from king grass at level of 3% (v/w) had better fermentative quality as compared to other silages. In addition, Fleigh point found in the present study was higher than the value of 41.7 in king grass silage as reported by Santoso *et al*. (2009), but it was lower than Fleigh point of 72.83 for alfalfa-maize silage mixture (Ozturk *et al*., 2006).

Chemical Composition and *in Vivo* Nutrient Digestibility

The OM content in rice crop residue-based silage was higher than rice straw (Table 4). This can be caused by rice crop residue-based silage in this experiment was added soybean curd and cassava containing OM 96.4% and 98.6%, respectively. Elephant grass containing proteins two times higher than rice straw and rice-based crop residue silage.

Table 5 shows DM, OM, and CP digestibility by goats. The DM digestibility value in goats fed elephant grass (A) and rice crop residue-based silage (B) was higher (P<0.01) than those fed rice straw (C). In this experiment, however, DM digestibility value in goats fed

Table 4. Chemical composition (%) of experimental feeds

	Feeds		
	A	B	C
Dry matter	20.4	90.5	21.7
Organic matter	89.3	77	82.3
Crude protein	14.9	7.1	7.5

Note: A= king grass; RS= rice straw; RCS= rice crop residue-based silage

Table 5. Nutrients digestibility (%) in goats fed elephant grass, rice straw and rice crop residue-based silage

Digestibility	Feeds			SEM	P
	A	B	C		
Dry matter	60.5^{A}	51.4^{B}	59.9^{A}	1.24	<0.01
Organic matter	65.9^{A}	55.0^{C}	60.7^{B}	0.88	<0.01
Crude protein	70.9^{A}	42.6^{C}	51.6^{B}	1.30	<0.01

Note: Means in the same row with different superscript differ significantly (P<0.01). A= Elephant grass; B= Rice straw; C= Rice crop residue based-silage.

elephant grass and rice-based crop residue silage was similar; suggesting that preservation of rice crop residue into silage is an effective way to improve the digestibility. Dry matter digestibility value in goats fed rice crop residue-based silage increased by 16.5% compared to fed rice straw. Organic matter and CP digestibility values in goats fed elephant grass were higher (P<0.01) than those fed a rice-based crop residue silage and rice straw. High OM and CP digestibility values in goats fed a elephant grass due to grass contains CP more than two times compared to hay and rice crop residue-based silage. These conditions cause the activity of rumen microbes such as bacteria and protozoa increased and subsequently followed by increases in the process of degradation and fermentation of feed. As compared to rice straw, the digestibility of OM and CP in goats fed rice crop residue-based silage increased by 10.4 and 21.1%, respectively. This result was supported by previous study by Ando *et al*. (2006) that addition of LAB increased the digestibility of dry matter, organic matter, and crude protein of guinea grass silage. However, Takahashi *et al*. (2005) reported that there was no differences in digestibility of DM, OM, and CP between sheep fed whole crop rice silage with or without the addition of fermented juice of epiphytic LAB from rice crop.

CONCLUSION

Addition of epiphytic LAB inoculant extracted from king grass to rice crop residue enhanced lactic acid concentration by 38.1% compared to control silage. Rice crop residue-based silage with addition of LAB inoculant from king grass had better fermentation quality than those ensiled with addition of LAB inoculant from rice crop residue. Feeding rice crop residue silage-based to goats significantly improved digestibility of DM, OM, and CP compared to rice straw.

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